Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order
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Introduction
Reelin required for inside-out formation of neocortical layers
Reeler mice: neocortical layers inverted, cells abnormally dispersed
Vldlr, Apoer2, Lrp9, Dab1 mutants: similar phenotype
Migrating neurons and radial glial cells are targets for reelin signaling (receptors, Dab1)
Key question: how does reelin affect inside-out formation of neocortical layers?
Possibilities
- Forms a graded signal from the MZ, instructing neurons to leave glial guides
- Allows neurons to pass cells during migration
Test: where does reelin need to be?
- Guidance or positional: required at specific site
- Permissive or enabling: source doesn’t matter
Cajal-Retzius Cells: produce reelin, located in MZ at feet of radial glia
Central role of CR?:
- Overexpression of BDNF (regulates reelin in CR cells): similar to reeler phenotype
- Low levels of reelin mRNA expressed in cortical primordium
- Ablation of CR disrupts cell migration to II/III (might affect other cell types)
- Meningeal cells that interact with CR contain neuronal guidance molecules
- Without reelin from CR: specific fragment of reelin provides partial rescue
Nestin-reelin
Expressed throughout VZ and probably radial glial cells
Radial glial expression not sufficient to rescue normal phenotype
CR cells express p73 (apoptosis): p73 mutants die but with normal cortical patterning

Results
Test if hem is source of CR cells:
Cre-loxP: Wnt3a-IRES-Cre crossed with R26R reporter (Bgal with Cre), immunohistochemistry
- internal ribosome entry site (IRES: circumvent the problems with transgenic variability and to knock-in a cDNA without destroying the targeted gene
- Wnt3a confined to hem -> Bgal cells as hem derivatives
Migrate to where CR located; predominate neocortex and hippocampus (not olfactory bulb or below ventrolateral line) -> complementary to CR localization

The cortical hem generates CR cells in the neocortical MZ
Distinguishing features of CR: reelin, calretinin, glutamate, no GABA, express p73, SDF1, CXCR4
In Wnt3a-IRES-Cre: MZ/Layer 1 cells express p73 and reelin, not GABA: hem-derived MZ cells are CR
Double-label (Bgal + reelin or p73)
- P73 Heterozygotes: 60% and 30% in medial and dorsolateral cortex Bgal
- P73 Homozygotes: 90% and 60%
Ongoing lateral migration from hem
Cells labeled with reelin alone born before Cre recombination, or are from outside the hem

Genetic ablation of the hem
Construct:
- cDNA for diptheria toxin, subunit a (dt-a) inserted into Wnt3a locus, stop codons flanked by loxP stop activation of toxin in Wnt3a-xneos-dt-a
- Cross with Emx1-IRES-Cre to remove stop codons and activate toxin (Emx1-dorsal telencephalon)
- Toxic activated in cortical hem
E10: almost complete hem ablation -> loss of Wnt3a, tissue lost
E12.5: hem derivatives (choroid plexus) missing as detected by Lmx1a; but some remain from caudal hem
Loss of CR cells in neocortical MZ

E12.5  WT: cerebral cortex covered in reln, p73 everywhere except olfactory bulb
       Double heterozygotes: both mostly absent from neocortical MZ

E13,15.5 WT: Reln-expressing CR form dense band in MZ
           Double heterozygotes: missing (expression, immunoreactivity)

E18.5  Double heterozygotes: few Reln cells in MZ: interneurons expressing Rln haven’t migrated
       Double heterozygotes: expression of SDF1 and CXCR4 (CR) absent

Conclusion: hem is origin of majority of neocortical CR cells

Prominence of other sources of migratory reelin-positive cells

Reln-expressing cells in ventral telencephalon, retrobulbar zone near olfactory bulb
CR cells that migrate into olfactory and rostromedial cortex arise from Dbx-1 progenitors near septum and boundary between dorsal and ventral telencephalon
Absence of hem-derived CR: Reln expressed ventral to neocortex and surrounding olfactory bulb; some move to CR cell-depleted cortex
Hypothesis: CR cells can be supplied from other sources
   Reln-expressing cells from other sources seen at E13.5, but not E15.5 or later
   Not typical CR cells
   Very few in lateral neocortical MZ and lateral neocortex
   Cells from other sources may be transient; hem-ablated cortex could be inhospitable for migration and survival

Low levels of reelin expression in cortical primordium (wt and mutant)

E12.5  faint band of Reln deep in cortical primordium
E13.5  just beneath pial surface
E15.5  lower IZ, SVZ
E18.5  individual cells could be resolved, overlap expression of Rorb (txn factor in IV and V)

Immunohistochemistry: no reelin protein in IZ/SVZ/CP (low protein levels, diffusion): mRNA levels low

Partition of the preplate in hem-ablated mice

Expect: in absence of CR cells from MZ: reeler phenotype
Test: Immunohistochemistry for calretinin, CSPGS
   WT: upper band marks CR cells of MZ, lower marks subplate
   Mutant: cortical plate (lower band) present

Preplate partitioning in reeler can be rescued by expression of reelin in VZ, but doesn’t rescue patterning
Predict: mutants would show inverted layers

Neocortical lamination in hem-ablated mice

Use molecular markers to distinguish layers
Mutants show defined, correctly-ordered layers
Gene expression sometimes more diffuse or out of position: modest migrational defects
Precise layer organization may require more CR cells
Defects in the caudal cerebral cortex after hem ablation
Rostral levels: near normal laminar pattern
Caudomedial: highly disorganized: Reln cells barely detectable but not a reeler defect
SCIP/Pouf3fl and Tbr1 not inverted but intermingled: caused by reduction of p73 (p73 mutants similar)

Discussion
Near absence of CR cells, neocortical layering is normal
Layer order doesn’t depend on abundant, localized source of reelin in MZ
Reelin from other sources can diffuse and compensate
Low level of Reln expressed below target layer: neurons must pass through -> not a detachment signal
CR cells can regulate the movement of other cell types like interneurons, influence dendritic morphogenesis